Elsevier Editorial System(tm) for European Journal of Obstetrics & Gynecology and Reproductive Biology
Manuscript Draft

Manuscript Number: EJOGRB-19-19302

Title: Markers of maternal cardiac dysfunction in pre-eclampsia and superimposed pre-eclampsia

Article Type: Full Length Article

Section/Category: Maternal-Fetal Medicine

Keywords: Pregnancy; pre-eclampsia; glycogen phosphorylase isoenzyme BB (GPBB); brain natriuretic peptide (BNP).

Corresponding Author: Dr. Frances Conti-Ramsden, MBBS MRCP

Corresponding Author's Institution: King's College London

First Author: Frances Conti-Ramsden, MBBS MRCP

Order of Authors: Frances Conti-Ramsden, MBBS MRCP; Carolyn Gill, PhD; Paul T Seed, MSc CStat; Kate Bramham, PhD; Lucy C Chappell, PhD; Fergus P McCarthy, PhD

Abstract: Objectives: To determine whether glycogen phosphorylase isoenzyme B (GPBB) and/or brain natriuretic peptide (BNP) concentrations are elevated in pre-eclampsia and superimposed pre-eclampsia (SPE), demonstrating cardiac ischaemia and strain.

Study design: A nested case-control study was performed using samples and clinical data available from a prospective pregnancy cohort. Four groups were selected: healthy pregnant controls (n=21), pre-eclampsia (n=19), pre-existing chronic hypertension (CHT) and/or chronic kidney disease (CKD) without (n=20) or with superimposed pre-eclampsia (SPE) (n=19). Plasma samples were taken at time of disease or the third trimester in controls.

Results: There was no significant difference in GPBB plasma concentrations between controls and pre-eclampsia (geometric mean (GM) [95% CI]: 4.74 [2.54-8.84] ng/mL vs 5.01 [2.58-9.74] ng/mL, p=0.90)), or between CHT and/or CKD and SPE (GM [95% CI]: 9.49 [4.93-18.25] ng/mL vs 10.24 [5.27-19.92] ng/mL, p=0.87). BNP plasma concentrations were significantly raised in women with pre-eclampsia compared to controls (GM [95% CI]: 31.83 [20.18-50.22] pg/mL vs 11.33 [7.34-17.51] pg/mL, p=0.001). Women with CKD, but not CHT, who developed SPE had elevated BNP concentrations. There were no significant differences in BNP concentration between women with comorbidity (CHT and/or CKD) and controls.

Conclusions: GPBB has a limited role as a biomarker in hypertensive disorders of pregnancy. BNP concentrations were elevated in pre-eclampsia compared to controls. This suggests cardiac strain at the time of pre-eclampsia. Further studies are needed to examine whether BNP can identify women at increased risk of cardiovascular disease.

Suggested Reviewers: Baskaran Thilaganathan
St George's University of London
basky@pobox.com
Expert in field.

Jenny Myers
University of Manchester
Jenny.Myers@manchester.ac.uk
Expert in the field.

Opposed Reviewers:

Markers of maternal cardiac dysfunction in pre-eclampsia and superimposed pre-eclampsia

Authors: Frances Conti-Ramsden MBBS Academic Clinical Fellow[1], Carolyn Gill PhD BRC Research Assistant[1], Paul T Seed MSc CStat Senior Lecturer in Medical Statistics[1], Kate Bramham PhD Clinical Senior Lecturer in Nephrology[2], Lucy C Chappell PhD NIHR Research Professor in Obstetrics[1], Fergus P McCarthy PhD Clinical Senior Lecturer in Obstetrics and Gynaecology[1,3].

This project was undertaken at the Department of Women and Children's Health, King's College London, and was funded by Tommy's Charity UK and the NIHR Biomedical Research Centre at Guy's and St Thomas' NHS Foundation Trust and King's College London. FP McCarthy was funded by an NIHR Clinical Academic Fellowship. Paul T Seed is partly funded by Tommy's (Registered charity no. 1060508) and by CLAHRC South London (NIHR)

Author affiliations:

[1]Department of Women and Children's Health, School of Life Course Sciences, King's College London, London, UK.

[2]Department of Renal Medicine, Division of Transplantation Immunology and Mucosal Biology, King's College London, London, UK.

[3]The Irish Centre for Fetal and Neonatal Translational Research, University College Cork, Wilton, Ireland Cork, Ireland.

Corresponding author:

Dr Frances Conti-Ramsden

Email: fran.conti-ramsden@kcl.ac.uk

Tel: 0207 188 3639

Postal address: Department of Women and Children's Health, 10th Floor North Wing, St Thomas' Hospital, Westminster Bridge Road, London, SE1 7EH, UK.

Author emails: fran.conti-ramsden@kcl.ac.uk, carolyn.gill@kcl.ac.uk, paul.seed@kcl.ac.uk, kate.bramham@kcl.ac.uk, lucy.chappell@kcl.ac.uk, fergus.mccarthy@ucc.ie

Abstract: Markers of maternal cardiac dysfunction in pre-eclampsia and superimposed pre-eclampsia

Authors: Frances Conti-Ramsden MBBS Academic Clinical Fellow[1], Carolyn Gill PhD BRC Research Assistant[1], Paul T Seed MSc CStat Senior Lecturer in Medical Statistics[1], Kate Bramham PhD Clinical Senior Lecturer in Nephrology[2], Lucy C Chappell PhD NIHR Research Professor in Obstetrics[1], Fergus P McCarthy PhD Clinical Senior Lecturer in Obstetrics and Gynaecology[1,3].

Objectives: To determine whether glycogen phosphorylase isoenzyme B (GPBB) and/or brain natriuretic peptide (BNP) concentrations are elevated in pre-eclampsia and superimposed pre-eclampsia (SPE), demonstrating cardiac ischaemia and strain.

Study design: A nested case-control study was performed using samples and clinical data available from a prospective pregnancy cohort. Four groups were selected: healthy pregnant controls (n=21), pre-eclampsia (n=19), pre-existing chronic hypertension (CHT) and/or chronic kidney disease (CKD) without (n=20) or with superimposed pre-eclampsia (SPE) (n=19). Plasma samples were taken at time of disease or the third trimester in controls.

Main outcome measures: Plasma concentrations of GPBB and BNP.

Results: There was no significant difference in GPBB plasma concentrations between controls and pre-eclampsia (geometric mean (GM) [95% CI]: 4.74 [2.54-8.84] ng/mL vs 5.01 [2.58-9.74] ng/mL, p=0.90)), or between CHT and/or CKD and SPE (GM [95% CI]: 9.49 [4.93-18.25] ng/mL vs 10.24 [5.27-19.92] ng/mL, p=0.87). BNP plasma concentrations were significantly raised in women with pre-eclampsia compared to controls (GM [95% CI]: 31.83 [20.18-50.22] pg/mL vs 11.33 [7.34-17.51] pg/mL, p=0.001). Women with CKD, but not CHT,

who developed SPE had elevated BNP concentrations. There were no significant differences in BNP concentration between women with comorbidity (CHT and/or CKD) and controls.

Conclusions: GPBB has a limited role as a biomarker in hypertensive disorders of pregnancy. BNP concentrations were elevated in pre-eclampsia compared to controls. This suggests cardiac strain at the time of pre-eclampsia. Further studies are needed to examine whether BNP can identify women at increased risk of cardiovascular disease.

Keywords: Pregnancy, pre-eclampsia, glycogen phosphorylase isoenzyme BB (GPBB), brain natriuretic peptide (BNP).

Highlights

- Glycogen phosphorylase isoenzyme B is not a useful biomarker in pre-eclampsia
- Brain natriuretic peptide (BNP) concentration is elevated in pre-eclampsia
- BNP concentration in SPE may be influenced by underlying comorbidity

INTRODUCTION

Women who experience hypertensive disorders of pregnancy have an increased risk of developing cardiovascular diseases including hypertension, stroke, and ischaemic heart disease later in life.[1–3] This risk is evident shortly after an affected pregnancy and persists for decades.[4] The mechanism underlying this association is not known, particularly whether women who develop hypertensive disorders are at higher risk pre-pregnancy and pregnancy unmasks their cardiovascular risk, or whether hypertensive disorders of pregnancy are an index event causing cardiovascular damage.[5] In keeping with the latter, it is plausible that the pathophysiological changes of pre-eclampsia, namely ischaemia and endothelial dysfunction,[6] cause irreversible cardiovascular impairment which contributes to the adverse cardiovascular outcomes later in life in these women.

GPBB is found in human heart and brain tissue and is an early marker of myocardial ischaemia, rising rapidly within the first hour of onset of chest pain in acute coronary syndromes.[7–12] BNP is primarily secreted by the left ventricle in response to cardiac chamber wall stretch as a result of systolic or diastolic dysfunction,[13–17] and concentrations have been shown to increase with greater levels of cardiac damage in the context of heart failure.[18] We hypothesised that subclinical cardiac ischaemia and dysfunction as demonstrated by elevated GPBB and BNP concentrations respectively occurs in women with pre-eclampsia and superimposed pre-eclampsia compared to controls and women with pre-existing chronic disease (chronic hypertension or chronic kidney disease). To investigate this, we measured concentrations of markers of cardiac ischaemia and strain: glycogen phosphorylase isoenzyme B (GPBB) and brain natriuretic peptide (BNP), at time of disease in women with pre-eclampsia or superimposed pre-eclampsia compared to controls.

METHODS

Study design

A nested case-control study was performed using samples and clinical data available from a pregnancy cohort, full details of which have been published elsewhere.[19] In brief, the PEACHES study investigated predictive and diagnostic biomarkers for pre-eclampsia in women with pre-existing disease (chronic kidney disease (CKD) or chronic hypertension (CHT)), with a comparator control group also recruited. Between June 2009 and September 2013, pregnant women with chronic kidney disease (CKD) or chronic hypertension (CHT) and healthy controls from two academic health science centres were prospectively enrolled between 20^{+0} and 36^{+6} weeks' gestation. All participants completed written informed consent. Venous blood was drawn in EDTA tubes between one and four times during pregnancy, and samples (plasma and serum) were centrifuged at 1,400 *x g* for 10 minutes at 4°C then stored at -80°C. Outcome data were recorded by case note review following delivery. Definitions were based on the International Society for Study of Hypertension in Pregnancy guidelines,[20] and are available in Supplementary Table 1. Ethical approval was given by the National Research Ethics Service (11/LO/1776). All study procedures were performed in line with the Declaration of Helsinki.

We selected four groups of pregnant women from the study: healthy women who did not develop pre-eclampsia (control group), women without pre-existing disease who developed pre-eclampsia (pre-eclampsia group), women with CKD and/or CHT who did not develop pre-eclampsia (CKD and/or CHT group), and women with CKD or CHT who developed superimposed pre-eclampsia (superimposed pre-eclampsia group). Samples assayed for

GPBB and BNP concentration were taken either at time of disease (pre-eclampsia and superimposed pre-eclampsia), or during the third trimester in controls.

Assay analyses

Plasma samples were assayed for GPBB concentration using Diagenics Dianeonatal® Glycogen phosphorylase Isoenzyme BB (GP-BB)-ELISA in-vitro diagnostic device kits (an Enzyme-linked immunosorbed assay for quantitative detection of GP-BB in human plasma), in line with the manufacturer's instructions. All assays were run in duplicate in a blinded randomised manner. Intra-assay coefficients of variation were 9.1% and 3.5%, and inter-assay coefficient of variation was 1.3%.

The Alere Triage® CardioRenal assay was used to assay BNP concentration in line with the manufacturer's instruction in a blinded randomised manner. It is a point of care fluorescence immunoassay which uses fluorescently labelled recombinant murine monoclonal antibodies. The assay specifically quantifies BNP concentration between the range 5-5000 pg/mL. The coefficient of variation on plasma controls were determined from the manufacturers leaflet and were 9.2% and 13.9% at concentrations of 78 and 3450 pg/mL respectively.

Statistical analysis

Data analysis was performed in Stata versions 14.2-15.1 (StataCorp, College Station, Texas). GPBB and BNP concentrations were compared between main groups, combined groups and subgroups. Standard distribution plots were used to assess normality of data and logarithmic transformation was applied for formal testing of differences using linear

regression of the transformed values with group differences expressed as ratios of the geometric mean (GM). Due to censoring of values less than the lower limit of detection or above the upper limit, interval regression was used.[21] Demographic characteristics and pregnancy outcomes between groups were compared using unpaired t-tests and Chi-squared tests.

RESULTS

Baseline variables and pregnancy outcomes

Seventy-nine women were identified from the PEACHES cohort: 21 controls, 19 with pre-eclampsia, 20 with CHT and/or CKD and 19 with superimposed pre-eclampsia (SPE). The demographic and clinical characteristics and pregnancy outcomes of the four groups are described in Tables 1 and 2. Compared to controls, women who developed pre-eclampsia had on average a higher body mass index (BMI), and were more likely to have a preterm delivery, lower birthweight infants, and more likely to deliver by Caesarean section. Compared to women with pre-existing CHT and/or CKD, women who developed SPE were also more likely to have preterm delivery, lower birthweight infants, and deliver by Caesarean section.

GPBB plasma concentrations in pre-eclampsia and superimposed pre-eclampsia

No significant difference was observed in GPBB plasma concentrations between the pre-eclampsia and control groups (geometric mean (GM) [95% CI]: 5.01 [2.58-9.74] ng/mL vs 4.74 [2.54-8.84] ng/mL; ratio GMs 1.06 [0.43-2.63], p=0.90), or between the SPE and CHT and/or CKD groups (when pooled across high-risk categories) (GM [95% CI]: 10.24 [5.27-19.92] ng/mL vs 9.49 [4.93-18.25] ng/mL; ratio GMs 1.08 [0.42-2.74], p=0.87). A box plot of

GPBB concentration in the sub-groups is shown in Figure 1. Comparison of GPBB concentrations in women with comorbidities against controls was undertaken. Considering women with CHT, CKD and CHT and CKD as separate groups, only women with CHT had elevated GPBB concentrations compared to controls (geometric mean (GM) [95% CI]: 19.84 [6.19-63.58] ng/mL vs 4.74 [2.54-8.84] ng/mL; ratio GMs 4.19 [1.12-15.70], p = 0.034); however, this difference was no longer significant once adjustments were made for maternal age, ethnicity and BMI (ratio GMs 3.54 [0.87-14.39], p = 0.077). In addition, in the regression model adjusted for maternal age, BMI, ethnicity, and pre-eclampsia neither CKD (p=0.172) nor CHT (p=0.099) diagnoses considered as separate variables had a significant effect on GPBB concentration.

Box plots for pooled groups are shown in Supplementary Figure 1. Adjusting for maternal age, ethnicity and BMI in regression models did not alter results (pre-eclampsia v control: ratio GMs 1.31 [0.51-3.32], p=0.57, SPE v CHT and/or CKD: ratio GMs 1.31 [0.53-3.22], p=0.56). Ethnicity was noted to be highly significant in this model. Black women had a relative reduction in GPBB concentration of -68.9% (95% confidence interval -85.7% to -32.5%) compared to white women after adjustment for maternal age, BMI, comorbidity and pre-eclampsia status.

BNP plasma concentration in pre-eclampsia and superimposed pre-eclampsia

BNP plasma concentrations were significantly raised in the pre-eclampsia group compared to the control group (GM [95% CI]: 31.83 [20.18-50.22] pg/mL vs 11.33 [7.34-17.51] pg/mL; ratio GMs 2.81 [1.50-5.27], p=0.001). This result became more significant after adjustment for maternal age, ethnicity and BMI (ratio GM 3.39 [1.78-6.47], p<0.001). No significant

differences were observed between the pooled groups of superimposed pre-eclampsia and CHT and /or CKD groups (GM [95% CI]: 20.66 [12.40-34.44] pg/mL vs 16.06 [9.83-26.23] pg/mL; ratio GMs 1.29 [0.63-2.61], p=0.486), and this was unchanged by adjusting for maternal age, ethnicity and BMI (ratio GMs 1.28 [0.66-2.50], p=0.461). A box plot of BNP concentration in the sub-groups is shown in Figure 2, with pooled groups shown in Supplementary Figure 2. When women with CHT, CKD and CHT/CKD were considered individually, none had significantly elevated BNP concentrations compared to controls (ratio GMs [95% CI] of CHT v control: 0.99 [0.40-2.49]; p = 0.989, CKD v control: 1.37 [0.57-3.28]; p=0.484, CHT and CKD v control: 2.00 [0.84-4.76]; p=0.116), and these findings were not altered by adjustment for maternal age, BMI and ethnicity.

Interval regression adjusted for maternal age, ethnicity and BMI was performed. Adjusted ratios of the geometric mean for the subgroups are shown in Table 3. In the CKD subgroup and CKD and CHT subgroup SPE was associated with elevated BNP concentrations (but not in the CHT group). Interaction tests were performed between pre-eclampsia diagnosis and CKD (p = 0.44) and between pre-eclampsia diagnosis and CHT (p <0.01), consistent with these results. CKD stage had no effect on BNP concentration in women with CKD (p=0.676, with adjustment for maternal age, BMI, ethnicity, presence of SPE or comorbid CHT).

DISCUSSION

In this study BNP concentrations were elevated in cases of pre-eclampsia compared to controls. BNP concentrations were also elevated in women with CKD with or without co-existent CHT but not CHT alone who had superimposed pre-eclampsia. GPBB concentrations were not elevated in pre-eclampsia or superimposed pre-eclampsia compared with controls,

but were lower in Black compared to White women. Our findings suggest cardiac strain at the time of pre-eclampsia, and evidence of differences in cardiac pathophysiology during SPE in women with CKD compared to CHT. We found no evidence of subclinical cardiac ischaemia as demonstrated by GPBB concentration in pre-eclampsia or SPE and a limited role for the use of GPBB in the evaluation of hypertensive disorders of pregnancy.

The limitations of our study include the relatively small numbers per group, which may have limited the power to detect differences between groups. Although there were differences in timing of samples between groups (time of disease for pre-eclampsia/SPE compared to third trimester for controls) which could also have attributed to variability in GPBB and BNP concentrations and confounded the results, data reported by Lee et al., [22] on 32 pregnant women with serial GPBB measures in each trimester reported no differences in GPBB concentration with advancing gestation. Similarly, serial BNP sampling in normal pregnancies have reported no variation with advancing gestational age.[23]

Our study differs with the findings of Lee et al., [22] which reported GPBB concentrations being elevated in preterm pre-eclampsia compared to gestational-age matched controls, and McCarthy et al., [24] which reported GPBB concentrations were elevated in women with term pre-eclampsia and small-for-gestational age pregnancies at time of disease compared to controls. All three studies used a Diagenics ELISA assay although the exact assay used differed between studies (Dianeonatal in our study, Diacordon in study by McCarthy et al., not specified in study by Lee et al.). Another potential explanation for the difference in findings is that the demographic characteristics of the women included in these studies differs; the SCOPE cohort reported by McCarthy et al. was 100% White, the study

population in Lee et al. was approximately 90% Black, whilst we had a mixed population. However, whilst we did demonstrate that GPBB levels are lower in Black than White women, adjusting for ethnicity did not alter our findings.

Several small studies have investigated concentrations of cardiac troponin I, (the most widely used marker of cardiac ischaemia in clinical practice), in women with pre-eclampsia with conflicting results.[25,26] In a systematic review, five out of nine studies found cardiac troponin concentrations were raised in pre-eclampsia, but studies were of poor quality.[27] Our study suggests there is no significant subclinical cardiac ischaemia at the time pre-eclampsia; however, further high quality studies combining biomarkers and echocardiographic variables are necessary.

Our finding of elevated BNP concentration in pre-eclampsia compared to controls is consistent with other small studies. In a systematic review of BNP concentrations in women with pre-eclampsia compared to pregnant controls the majority of studies reported raised BNP concentrations although small sample size, heterogeneity and poor study quality precluded a quantitative analysis.[28] Our study is the first to report BNP concentrations in women with superimposed pre-eclampsia, and whilst there were no significant differences between groups overall, in a post-hoc subgroup analysis BNP concentrations were significantly elevated in women with CKD or CKD and CHT who developed pre-eclampsia. Given the small numbers in our study this result must be interpreted with caution; however, it is plausible that cardiac pathophysiology in SPE may differ depending on the underlying comorbidity and that women with CHT alone have a wider spectrum of disease, including those with minimal cardiac dysfunction.

The clinical significance of the raised BNP concentrations in our study are uncertain as we did not have access to vascular function measures or echocardiography as part of the original study. In a study comparing 40 women with pre-eclampsia to 35 normotensive controls mean (standard deviation) BNP concentration was 37.1 (10.0) pg/mL in the pre-eclampsia group compared to 21.5 (8.0) pg/mL in controls, and there was a linear association between BNP concentration and left ventricular end-diastolic volume.[29] Similar associations between BNP concentration and echocardiographic features of left ventricular diastolic dysfunction and depression of cardiac output are suggested by other small studies.[28] Therefore larger studies and studies that prospectively evaluate the relationship between cardiac biomarkers and adverse cardiovascular outcomes following hypertensive disorders of pregnancy are warranted.

CONCLUSION

Currently there is insufficient evidence to support GPBB testing as a useful diagnostic or prognostic test in pre-eclampsia. We have replicated findings from other small studies that BNP concentrations are elevated in pre-eclampsia, suggesting subclinical cardiac strain at time of disease. Larger studies are required to confirm this finding and investigate whether BNP and other cardiac biomarkers at time of disease in pre-eclampsia and superimposed pre-eclampsia can be used to identify women at high risk of long-term cardiovascular disease.

Acknowledgements

The authors would like to acknowledge all the pregnant women who participated in the study; research assistants and midwives in the Pregnancy Complications team at St Thomas' Hospital who were involved in patient recruitment and outcome collection; and Diagenics for providing enzyme kits.

Conflict of interests

Declarations of interest: none.

Funding

This project was funded by Tommy's Charity UK and the NIHR Biomedical Research Centre at Guy's and St Thomas' NHS Foundation Trust and King's College London. FP McCarthy was funded by an NIHR Clinical Academic Fellowship. Paul T Seed is partly funded by Tommy's (Registered charity no. 1060508) and by CLAHRC South London (NIHR)

Authorship

FM, LCC and KB conceived the idea for the study. Sample processing and analysis was managed by CG. Data extraction, manipulation and analysis was performed by FCR and PS. The first draft of the manuscript was written by FCR, and was subsequently revised and approved by all other authors.

References

1. Bellamy L, Casas J-P, Hingorani AD, Williams DJ. Pre-eclampsia and risk of

cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ*. 2007;335(7627):974. doi:10.1136/bmj.39335.385301.BE.

2. Brown MC, Best KE, Pearce MS, Waugh J, Robson SC, Bell R. Cardiovascular disease risk in women with pre-eclampsia: Systematic review and meta-analysis. *Eur J Epidemiol*. 2013;28(1):1-19. doi:10.1007/s10654-013-9762-6.

3. Lykke JA, Langhoff-Roos J, Sibai BM, Funai EF, Triche EW, Paidas MJ. Hypertensive pregnancy disorders and subsequent cardiovascular morbidity and type 2 diabetes mellitus in the mother. *Hypertension*. 2009;53(6):944-951. doi:10.1161/HYPERTENSIONAHA.109.130765.

4. Behrens I, Basit S, Melbye M, et al. Risk of post-pregnancy hypertension in women with a history of hypertensive disorders of pregnancy: nationwide cohort study. *BMJ*. 2017;358:j3078. doi:10.1136/bmj.j3078.

5. Melchiorre K, Sharma R, Thilaganathan B. Cardiovascular implications in preeclampsia: An overview. *Circulation*. 2014;130(8):703-714. doi:10.1161/CIRCULATIONAHA.113.003664.

6. Uzan J, Carbonnel M, Piconne O, Asmar R, Ayoubi JM. Pre-eclampsia: Pathophysiology, diagnosis, and management. *Vasc Health Risk Manag*. 2011;7(1):467-474. doi:10.2147/VHRM.S2018.

7. Rabitzsch G, Mair J, Lechleitner P, et al. Isoenzyme BB of glycogen phosphorylase b and myocardial infarction. *Lancet*. 1993;341(8851):1032-1033. doi:10.1016/0140-6736(93)91129-A.

8. Mair J, Puschendorf B, Smidt J, et al. Early release of glycogen phosphorylase in patients with unstable angina and transient ST-T alterations. *Br Heart J*. 1994;72(2):125-127. http://heart.bmj.com/content/heartjnl/72/2/125.full.pdf.

9. Rabitzsch G, Mair J, Lechleitner P, et al. Immunoenzymometric assay of human glycogen phosphorylase isoenzyme BB in diagnosis of ischemic myocardial injury. *Clin Chem*. 1995;41(7):966-978. http://clinchem.aaccjnls.org/content/41/7/966.long.

10. Mair J. Glycogen phosphorylase isoenzyme BB to diagnose ischaemic myocardial damage. In: *Clinica Chimica Acta*. Vol 272. Elsevier; 1998:79-86. doi:10.1016/S0009-8981(97)00254-4.

11. Peetz D, Post F, Schinzel H, et al. Glycogen phosphorylase BB in acute coronary syndromes. *Clin Chem Lab Med*. 2005;43(12):1351-1358. doi:10.1515/CCLM.2005.231.

12. Bozkurt S, Kaya EB, Okutucu S, Aytemir K, Coskun F. The diagnostic and prognostic value of first hour glycogen phosphorylase isoenzyme BB. *Cardiol J*. 2011;18(5):496-502. https://journals.viamedica.pl/cardiology_journal/article/view/21198.

13. Nakao K, Mukoyama M, Hosoda K, et al. Biosynthesis, secretion, and receptor selectivity of human brain natriuretic peptide. *Can J Physiol Pharmacol*. 1991;69(10):1500-1506. doi:10.1139/y91-225.

14. Yasue H, Yoshimura M, Sumida H, et al. Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. *Circulation*. 1994;90(1):195-203. doi:10.1161/01.CIR.90.1.195.

15. Nakao K, Ogawa Y, Suga S, Imura H. Molecular biology and biochemistry of the natriuretic peptide system. I: Natriuretic peptides. *J Hypertens*. 1992;10(9):907-912. http://www.ncbi.nlm.nih.gov/pubmed/1328371.

16. Mukoyama M, Nakao K, Hosoda K, et al. Brain natriuretic peptide as a novel cardiac hormone in humans: Evidence for an exquisite dual natriuretic peptide system, atrial

natriuretic peptide and brain natriuretic peptide. *J Clin Invest*. 1991;87(4):1402-1412. doi:10.1172/JCI115146.

17. Hosoda K, Nakao K, Mukoyama M, et al. Expression of brain natriuretic peptide gene in human heart. Production in the ventricle. *Hypertension*. 1991;17(6 Pt 2):1152-1155. doi:10.1161/01.HYP.17.6.1152.

18. Groenning BA, Nilsson JC, Sondergaard L, Kjaer A, Larsson HBW, Hildebrandt PR. Evaluation of impaired left ventricular ejection fraction and increased dimensions by multiple neurohumoral plasma concentrations. *Eur J Heart Fail*. 2001;3(6):699-708. doi:10.1016/S1388-9842(01)00181-7.

19. Bramham K, Seed PT, Lightstone L, et al. Diagnostic and predictive biomarkers for pre-eclampsia in patients with established hypertension and chronic kidney disease. *Kidney Int*. 2016;89(4):874-885. doi:10.1016/j.kint.2015.10.012.

20. Tranquilli AL, Dekker G, Magee L, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: A revised statement from the ISSHP. *Pregnancy Hypertens*. 2014;4(2):97-104. doi:10.1016/j.preghy.2014.02.001.

21. Amemiya T. Regression Analysis when the Dependent Variable Is Truncated Normal. *Econometrica*. 1973. doi:10.2307/1914031.

22. Lee J, Romero R, Dong Z, et al. Glycogen phosphorylase isoenzyme BB plasma concentration is elevated in pregnancy and preterm preeclampsia. *Hypertension*. 2012;59(2):274-282. doi:10.1161/HYPERTENSIONAHA.111.177444.

23. Resnik JL, Hong C, Resnik R, et al. Evaluation of B-type natriuretic peptide (BNP) levels in normal and preeclamptic women. *Am J Obstet Gynecol*. 2005;193(2):450-454. doi:10.1016/j.ajog.2004.12.006.

24. McCarthy FP, Doyle A, Khashan AS, Kenny LC. Altered Maternal Plasma Glycogen

Phosphorylase Isoenzyme BB as a Biomarker for Preeclampsia and Small for Gestational Age. *Reprod Sci*. 2016;23(6):738-747. doi:10.1177/1933719115616495.

25. Fleming SM, O'Gorman T, Finn J, Grimes H, Daly K, Morrison JJ. Cardiac troponin I in pre-eclampsia and gestational hypertension. *Br J Obstet Gynaecol*. 2000. doi:10.1111/j.1471-0528.2000.tb11658.x.

26. Aydin C, Baloglu A, Cetinkaya B, Yavuzcan A. Cardiac troponin levels in pregnant women with severe pre-eclampsia. *J Obstet Gynaecol (Lahore)*. 2009. doi:10.1080/01443610903154598.

27. Pergialiotis V, Prodromidou A, Frountzas M, Perrea DN, Papantoniou N. Maternal cardiac troponin levels in pre-eclampsia: a systematic review. *J Matern Neonatal Med*. 2016. doi:10.3109/14767058.2015.1127347.

28. Afshani N, Moustaqim-Barrette A, Biccard BM, Rodseth RN, Dyer RA. Utility of B-type natriuretic peptides in preeclampsia: A systematic review. *Int J Obstet Anesth*. 2013;22(2):96-103. doi:10.1016/j.ijoa.2012.11.001.

29. Borghi C, Esposti DD, Immordino V, et al. Relationship of systemic hemodynamics, left ventricular structure and function, and plasma natriuretic peptide concentrations during pregnancy complicated by preeclampsia. *Am J Obstet Gynecol*. 2000. doi:10.1067/mob.2000.105684.

Table

Table 1. Maternal characteristics of control, pre-eclampsia, chronic hypertension (CHT) and/or chronic kidney disease (CKD) and superimposed pre-eclampsia groups.

	Controls	Pre-eclampsia	p-value	CHT and/or CKD	Superimposed pre-eclampsia	p-value
Number of women	21	19		20	19	
Demographic variables						
Age, y, median (IQR)	29 (24-33)	29 (25-36)	0.50	35 (26-38.5)	32 (28-36)	0.61
BMI, kg/m2, median (IQR)	22.6 (21.3-27.1)	29.1 (24.7-32.4)	**0.03**	28.3 (24.6-32.0)	28.7 (23.3-34.7)	0.57
Nulliparous, n (%)	16 (76.2)	14 (73.7)	0.86	10 (50.0)	9 (47.4)	0.87
Ethnicity						
White ethnicity, n (%)	15 (71.4)	7 (36.8)		11 (55.0)	7 (36.8)	
Black ethnicity, n (%)	5 (23.8)	9 (47.4)	0.08	7 (35.0)	8 (42.1)	0.47
Asian ethnicity, n (%)	1 (4.8)	1 (5.3)		0 (0)	1 (5.3)	
Other ethnicity, n (%)	0 (0)	2 (10.5)		2 (10.0)	3 (15.8)	
Smoking status						
Current smoking, n (%)	3 (14.3)	1 (5.3)		1 (5.0)	2 (10.5)	
Quit smoking, n (%)	1 (4.8)	2 (10.5)	0.56	1 (5.0)	3 (15.8)	0.44
Never smoked, n (%)	17 (81.0)	16 (84.2)		18 (90.0)	14 (73.7)	
Medical history						
Pre-gestational diabetes mellitus, n (%)	0 (0)	1 (5.3)	0.29	1 (5.0)	2 (10.6)	0.52
Chronic hypertension, n (%)	0 (0)	0 (0)	-	13 (65.0)	15 (78.9)	0.33
Chronic kidney disease	0 (0)	0 (0)		14 (70.0)	8 (42.1)	0.08
Stage 1	-	-		8 (40.0)	5 (26.3)	
Stage 2	-	-	-	5 (25.0)	1 (12.5)	
Stage 3	-	-		1 (5.0)	1 (12.5)	
Stage 4	-	-		0 (0)	1 (12.5)	

Abbreviations: IQR = interquartile range.

Table

Table 2. Pregnancy outcomes of control, pre-eclampsia, chronic hypertension (CHT) and/or chronic kidney disease (CKD) and superimposed pre-eclampsia groups. *Abbreviations:* IQR = interquartile range, SGA = small for gestational age.

	Controls	Pre-eclampsia	p-value	CHT and/or CKD	Superimposed pre-eclampsia	p-value
Number of women	21	19		20	19	
Neonatal outcomes						
Gestation at delivery, wk, median (IQR)	40.3 (39.9-41.0)	37.6 (34.7-38.3)	**<0.01**	39.0 (38.4-40.1)	36.6 (34.1-38.1)	**<0.01**
Preterm delivery <37/40, n (%)	1 (4.8)	8 (42.1)	**<0.01**	1 (5.0)	10 (52.6)	**<0.01**
Preterm delivery <34/40, n (%)	0 (0.0)	2 (10.5)	0.13	0 (0)	3 (15.8)	0.06
Intrauterine death, n (%)	0 (0)	0 (0)	-	0 (0)	0 (0)	-
Birth weight, g, median (IQR)	3300 (3000-3620)	2400 (1970-3120)	**<0.01**	3190 (3055-3395)	2500 (2000-3000)	**<0.01**
SGA (<10th customized birth weight centile)	5 (23.8)	7 (36.8)	0.37	3 (15.0)	8 (42.1)	0.06
SGA (<3rd customized birth weight centile)	0 (0)	3 (15.8)	0.06	2 (10.0)	4 (21.1)	0.34
Mode of delivery						
Spontaneous vaginal delivery, n (%)	19 (90.5)	4 (21.1)	**<0.01**	6 (30.0)	1 (5.3)	**0.04**
Assisted vaginal delivery, n (%)	1 (4.8)	2 (10.5)	0.49	4 (20.0)	1 (5.3)	0.17
Caesarean delivery, n (%)	1 (4.8)	13 (68.4)	**<0.01**	10 (50.0)	17 (89.5)	**<0.01**
GPBB and BNP sampling						
Gestational age at sampling, weeks, median (IQR)	34.4 (32.6-37.4)	36.0 (33.7-37.3)	0.15	37.1 (34.3-37.8)	33.0 (26.9-34.9)	**<0.01**

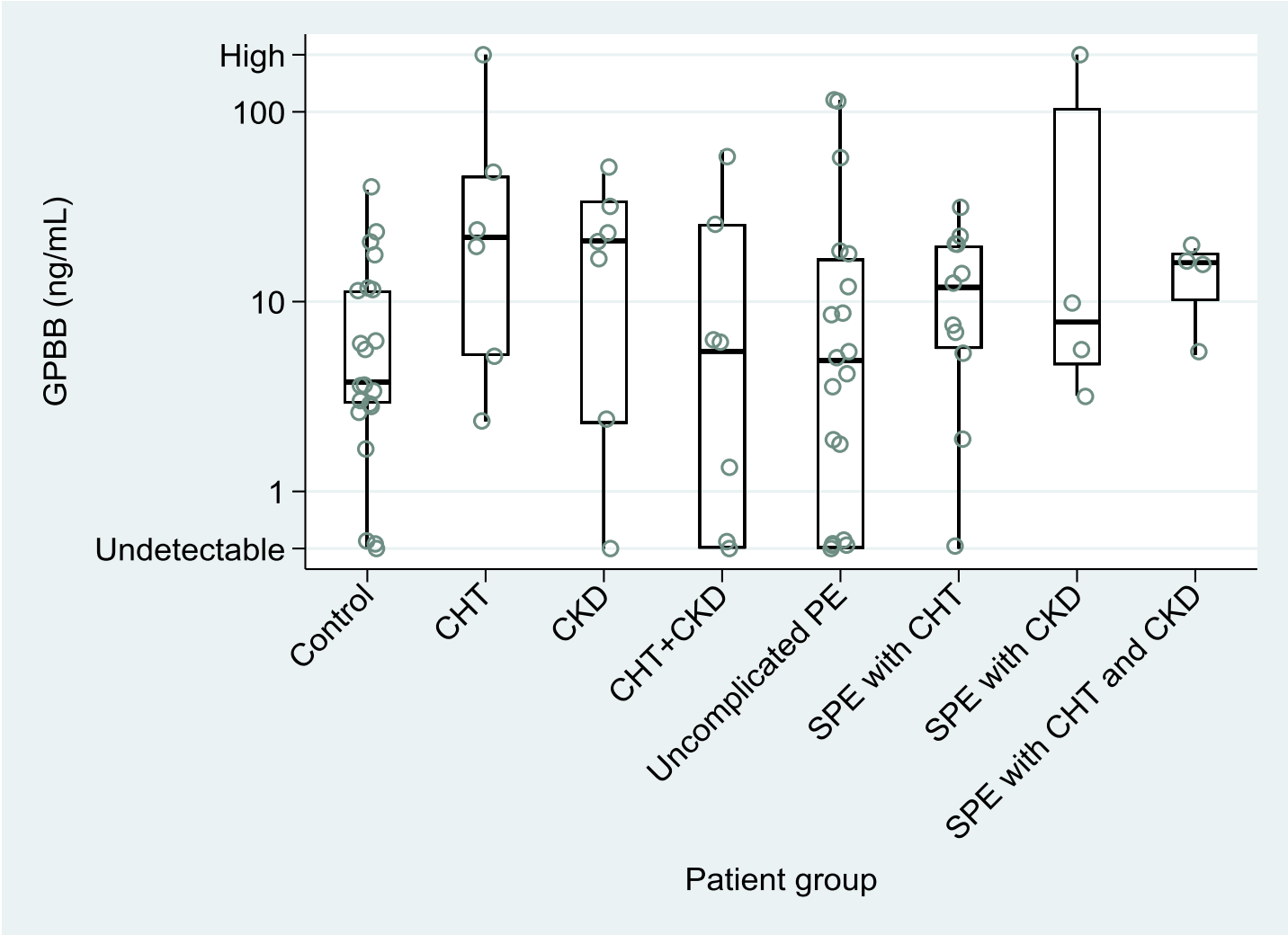
Table

Table 3. BNP concentrations with ratio of geometric means (GM) adjusted for maternal age, ethnicity and BMI comparing control, chronic hypertension (CHT), chronic kidney disease (CKD), and CHT and CKD groups with and without pre-eclampsia.

	BNP concentration (ng/mL)			
Subgroup	No (S)PE (GM [95% C.I.])	(S)PE (GM [95% C.I.])	Adjusted ratio of GM [95% C.I.]	p-value
Controls (n=21)	11.3 [7.34-17.5]	31.8 [20.2-50.2]	3.39 [1.78-6.47]	**<0.001**
CHT (n=17)	11.3 [5.02-25.3]	9.74 [5.20-18.3]	0.39 [0.11-1.48]	0.168
CKD (n=11)	15.5 [7.26-33.1]	98.0 [36.4-264]	9.48 [4.90-18.3]	**<0.001**
CHT and CKD (n=11)	22.7 [10.7-48.0]	31.3 [11.6-84.3]	3.30 [2.09-5.19]	**<0.001**

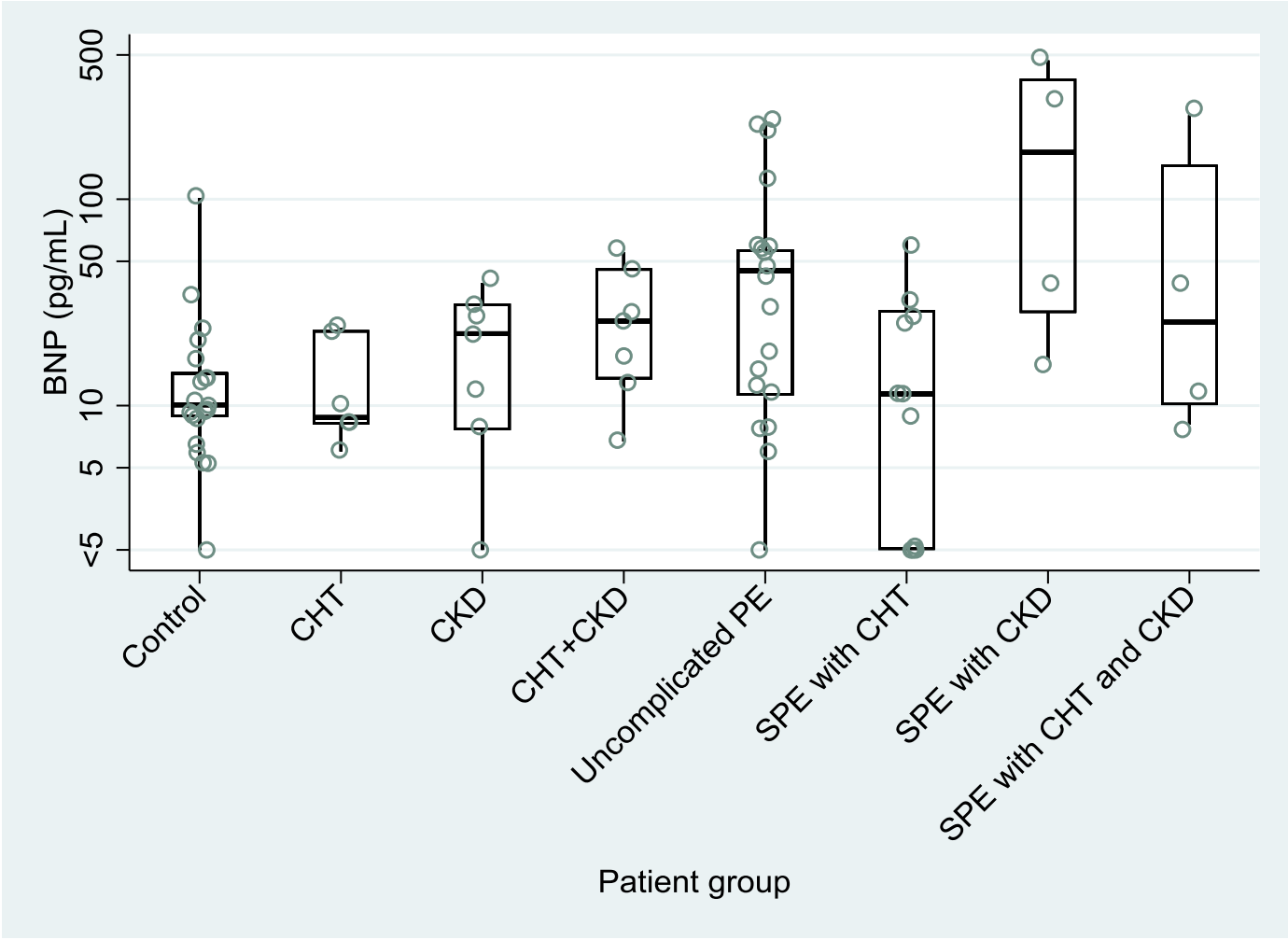
Figure

Figure 1. Box plots with overlaid log values of GPBB plasma concentration (ng/mL) in control, chronic hypertension (CHT), chronic kidney disease (CKD), CHT and CKD, pre-eclampsia (PE), and superimposed pre-eclampsia (SPE) groups.



Figure

Figure 2. Box plots with overlaid log values of BNP plasma concentration (pg/mL) in control, chronic hypertension (CHT), chronic kidney disease (CKD), CHT and CKD, pre-eclampsia (PE), and superimposed pre-eclampsia (SPE) groups.



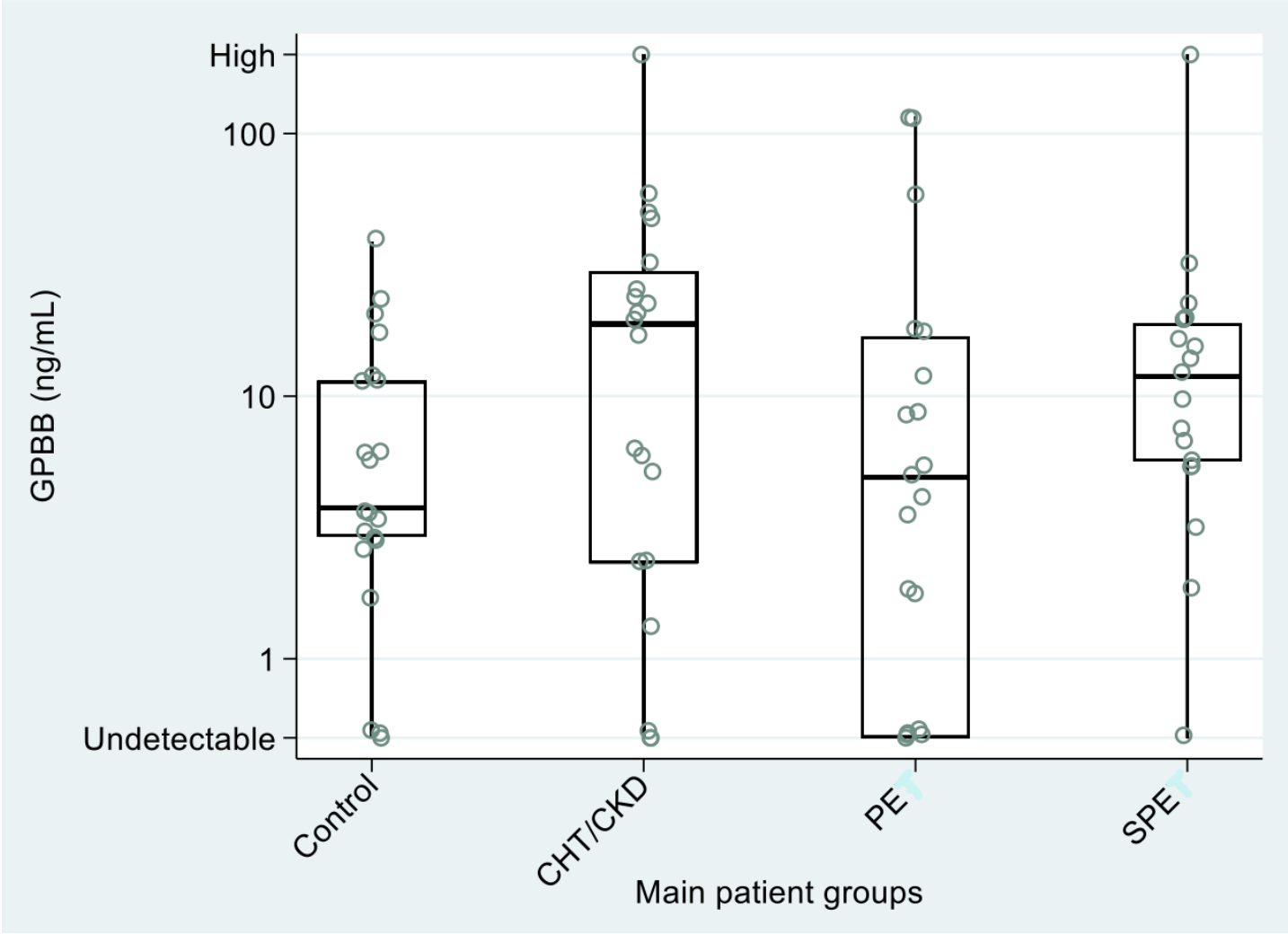
Figure

Supplementary Table 1: Definitions for study entry into PEACHES cohort.

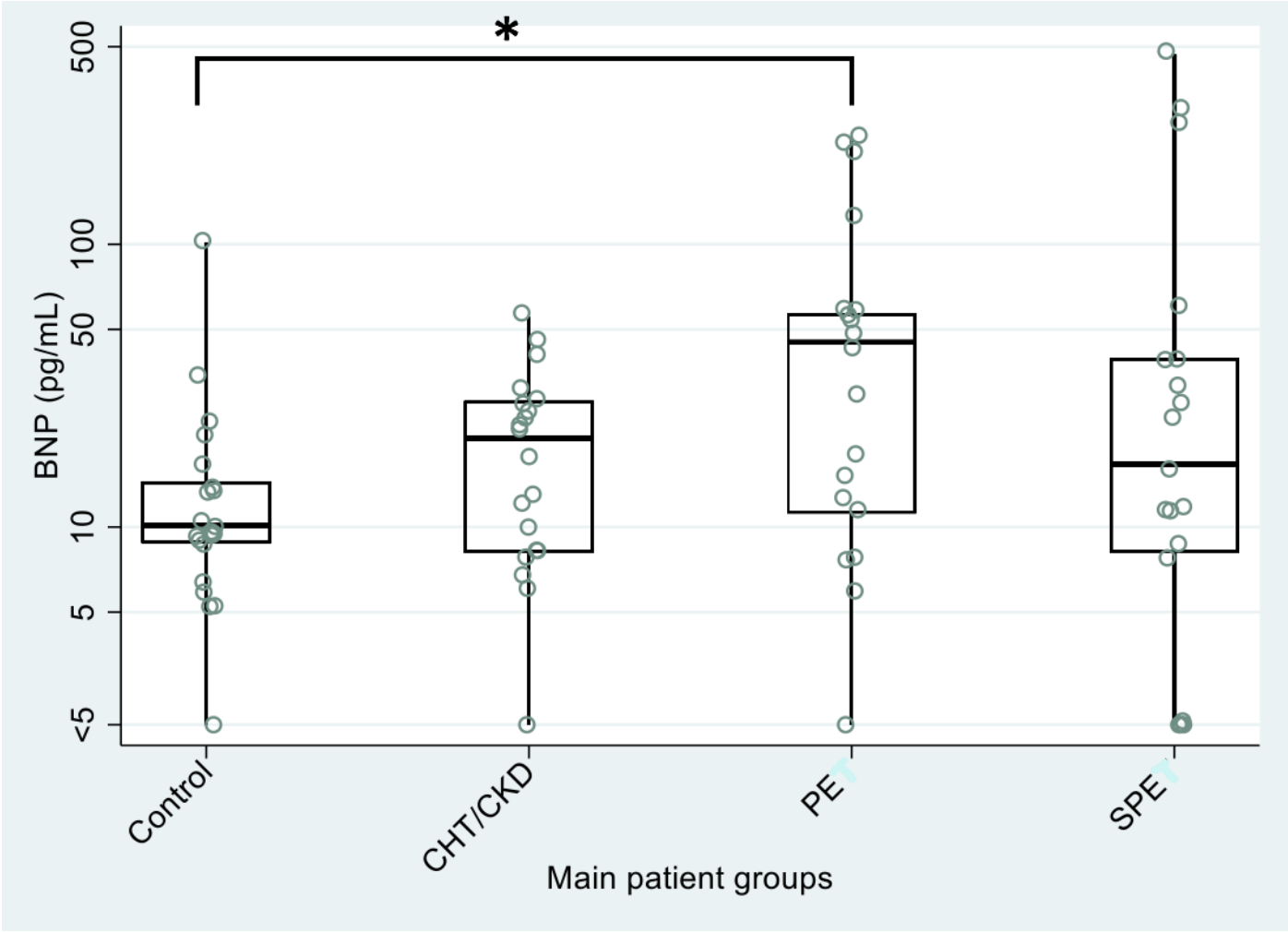
Definition	Criteria
Healthy control women	• No risk factors for pre-eclampsia • No history of pre-eclampsia, hypertension, diabetes, renal disease, connective tissue disease or anti-phospholipid antibody syndrome • Systolic blood pressure <140mmHg • Diastolic blood pressure <90mmHg • No protein on dipstick analysis of midstream urine • Not in labour
Gestational Hypertension	• Previously normotensive • Two recordings of systolic blood pressure ≥140mmHg or diastolic blood pressure ≥ 90mmHg greater than 4 hours apart • After 20 weeks' gestation • Not in labour
Pre-eclampsia	• Gestational Hypertension AND • Proteinuria of >300mg protein over 24 hours, (or protein:creatinine ratio of >30mg/mmol);
Superimposed pre-eclampsia *Hypertension already present*	• New onset of proteinuria >300mg protein over 24 hours, (or protein:creatinine ratio of >30mg/mmol); OR Additional features – severe persistent right upper quadrant pain or epigastric pain unresponsive to mediation or alanine transaminase > 71U/l or platelet count <100,000/µl or pulmonary oedema or new onset cerebral or visual disturbance
Superimposed pre-eclampsia *Proteinuria already present*	• Two recordings of systolic blood pressure ≥140mmHg or diastolic blood pressure ≥ 90mmHg greater than 4 hours apart OR • Additional features as listed above
Superimposed pre-eclampsia	• Development of severe hypertension (Systolic blood pressure ≥160mmHg or diastolic blood pressure ≥110mmHg)

Hypertension and proteinuria already present	AND • Greater than two fold increase in proteinuria above 300mg protein over 24 hours, (or protein:creatinine ratio of >30 mg/mmol); OR Additional features as listed above
Primary Hypertension	• Maternal diastolic blood pressure of 90mmHg or more before 20 weeks' gestation in the current pregnancy OR • Taking antihypertensive agents before 20 weeks' gestation OR • Taking antihypertensives prior to pregnancy • Secondary causes of hypertension excluded
Chronic Hypertension	• Primary or secondary causes of hypertension
Chronic Kidney Disease	• According to Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines pre-pregnancy {Guidelines, #50302} OR • Persistent proteinuria (>1+ or 30mg/mmol (protein creatinine ratio) before 20 weeks' gestation OR • Any recorded serum creatinine >70µmol before 20 weeks' gestation without risk factors for acute kidney injury;
Exclusion	• Women < 18 years old or >50 years old • Inability or unwillingness to give informed consent • Known HIV, Hepatitis B or C positive • Multi-fetal Pregnancy

Supplementary Figure 1. Box plots with overlaid log values of GPBB plasma concentration (ng/mL) in control, chronic hypertension (CHT) and/or chronic kidney disease (CKD), pre-eclampsia (PE) and superimposed pre-eclampsia (SPE) groups.



Supplementary Figure 2. Box plots with overlaid log values of BNP plasma concentration (pg/mL) in control, chronic hypertension (CHT) and/or chronic kidney disease (CKD), pre-eclampsia (PE) and superimposed pre-eclampsia (SPE) groups. *p value <0.05.



***Conflict of Interest**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
Methods		
Study design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
		(*b*) For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) If applicable, explain how matching of cases and controls was addressed
		(*e*) Describe any sensitivity analyses
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		(b) Indicate number of participants with missing data for each variable of interest
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.